

Shipbuilding docks as experimental systems for realistic assessments of anthropogenic stressors on marine organisms

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Abstract:	<p>Empirical investigations of the impacts of anthropogenic stressors on marine organisms are typically performed under controlled laboratory conditions or via offshore experiments with realistic (but uncontrolled) environmental variation. Both approaches have merits, but laboratories generally fail to recreate natural environments or stressor-fields, and offshore studies are often compromised by confounding factors. We pioneered the use of a flooded shipbuilding dock to study realistic exposure to stressors and their impacts on intra- and interspecific responses of animals. Shipbuilding docks allow careful study of groups of known animals, including evaluation of their behavioral interactions, while enabling full control of the stressor and many environmental conditions. We showcase this approach for assessing impacts of pile-driving noise on fishes, and propose its use to study other anthropogenic stressors, including chemicals and ocean warming. Results from shipbuilding-dock studies could allow improved parameterization of predictive models relating to environmental risks and population consequences of anthropogenic stressors.</p>

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Draft Manuscript

**Shipbuilding docks as experimental systems for realistic assessments of
anthropogenic stressors on marine organisms**

Short title: Assessing realistic impacts of stressors

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24 Abstract

25 Empirical investigations of the impacts of anthropogenic stressors on marine organisms are
26 typically performed under controlled laboratory conditions or via offshore experiments with
27 realistic (but uncontrolled) environmental variation. Both approaches have merits, but
28 laboratories generally fail to recreate natural environments or stressor-fields, and offshore
29 studies are often compromised by confounding factors. We pioneered the use of a flooded
30 shipbuilding dock to study realistic exposure to stressors and their impacts on intra- and
31 interspecific responses of animals. Shipbuilding docks allow careful study of groups of
32 known animals, including evaluation of their behavioral interactions, while enabling full
33 control of the stressor and many environmental conditions. We showcase this approach for
34 assessing impacts of pile-driving noise on fishes, and propose its use to study other
35 anthropogenic stressors, including chemicals and ocean warming. Results from shipbuilding-
36 dock studies could allow improved parameterization of predictive models relating to
37 environmental risks and population consequences of anthropogenic stressors.

38 Introduction

39 The human population and associated industrial activity has greatly increased during recent
40 decades, resulting in a rise in anthropogenic (man-made) pollution in terrestrial and aquatic
41 environments. In the marine environment this has led to changes in the physicochemistry of
42 our oceans. These changes include ocean warming ($\sim 0.11^{\circ}\text{C}$ global water warming per
43 decade of the top 75 m since 1971; IPCC 2014), increased seawater acidity (ocean surface
44 water increased 0.1 pH units compared to pre-industrial levels; Raven et al. 2005), regional
45 changes in ocean salinity as a consequence of global warming (>0.1 p.s.u. salinity increase in
46 the top 500 m in high-evaporation regions in four decades in the Atlantic Ocean; Curry et al.
47 2003), and increased levels of ocean noise (e.g. 3.3 dB per decade since 1950 in the
48 Northeast Pacific Ocean; Frisk 2012).

49 A range of human activities, including fossil-fuel consumption, resource extraction,
50 construction, transportation and waste disposal generate pollution, and many of these
51 activities and their potential impacts are expected to increase in the coming decades
52 (Gattuso et al. 2015, Slabbekoorn Hans et al. 2010). Environmental stressors generated by
53 human disturbance - hereafter referred to as 'anthropogenic stressors' - can impact
54 negatively on marine organisms (e.g. Palstra et al. 2006) and some have been linked to
55 population declines (Wada et al. 2013). Furthermore, impacts of marine contaminants can
56 affect human health through consumption of fish from polluted waters (Foran et al. 2005).
57 The increase in anthropogenic stressors in the oceans requires better understanding of
58 impacts and consequences of current and predicted future stressor levels on marine
59 organisms.

60 In this paper, we appraise the current methods used to study impacts of
61 anthropogenic stressors on marine animals. We then introduce a novel approach – flooded

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62 shipbuilding docks – to studying impacts of man-made stressors on marine organisms and
63 discuss merits and limitations of this approach compared to laboratory and offshore setups.
64 We then showcase an example of dock usage to study impacts of anthropogenic noise on
65 marine animals, evaluate strengths and weaknesses of this approach, and propose that a
66 dock approach could be used to study impacts of other anthropogenic stressors on marine
67 animals, including chemicals, eutrophication, salinity and ocean warming. Finally, we
68 provide guidance on how to conduct such experiments using a dock setup, highlight
69 opportunities and challenges, and propose questions this novel approach could be used to
70 address.

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72 **Current empirical methods used to study impacts of anthropogenic stressors on marine**
73 **animals**

74 To date, our understanding of the impact of anthropogenic stressors on marine animals is
75 derived from a combination of laboratory experiments and offshore studies. Here we
76 compare these approaches and assess their merits and limitations (see Table 1 for a
77 summary).

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79 **Laboratory experiments**

80 Experiments conducted in laboratory conditions have been effective in testing potential
81 impacts of a range of environmental stressors on individual organisms, including ocean
82 warming (Scott and Johnston 2012) and ocean acidification (reviewed in: Fabry et al. 2008).
83 Such studies have helped to decipher underlying mechanisms, identify stressor thresholds
84 and have highlighted critical consequences of these stressors. Generally, laboratory studies

allow for tight control of potential confounding factors and enable investigations that are difficult (or impossible) to carry out in the field. An example of this includes long-term studies performed under well-defined conditions. However, laboratory studies generally fail to capture environmental complexity (Taylor et al. 2015), are unlikely to recreate natural conditions of the 'stressor experience' (Slabbekoorn H. 2016), and typically use small aquaria (for the purpose of this paper an aquarium of 200 L is envisaged when comparing methods). Some of the greatest challenges for laboratory-based experiments are to assess impacts of stressors on individual phenotypes due to general phenotypic complexity, which can be influenced strongly, for example, by the social context; this is rarely accounted for in the laboratory. Additionally, stressors can also impact animals through disrupting interactions between individuals or between species, something rarely considered in the laboratory.

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98 **Offshore experiments using mesocosms**

Studies that investigate impacts of anthropogenic stressors on animals in offshore locations, defined as any study located in the sea away from the shore, typically use mesocosms; an experimental system enclosing the study organisms. Such studies have showcased the possible impacts of several anthropogenic stressors, including ocean acidification (e.g. Kline et al. 2012) and chemicals (making use of existing contaminated locations: Berge and Brevik 1996). The main advantages of using mesocosms for offshore studies are that wild animals can be tested and investigated in their natural – albeit enclosed – environment, which potentially captures local physicochemical and biotic complexity. In addition, offshore mesocosms allow the study of stressors that are not possible to study without enclosures.

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108 For example, investigating impacts of ocean acidification using small-scale (<2 m³) enclosed
109 units that can be placed upon the ocean floor (reviewed in Gattuso et al. 2014). Such sealed
110 units ensure continuous stressor exposure during the experiment and recapture of the
111 study animals following the experiments. However, their small size precludes testing larger
112 animals, or those that require larger living space, and might prevent studying conspecific
113 and interspecific interactions as well as stressor impacts at a community level. As with any
114 enclosure, care must be taken that the enclosure size permits natural behavior and does not
115 impair the health of the study animals. Other disadvantages of offshore studies using
116 mesocosms include logistical complexity and high expense compared to equivalent
117 experiments in the laboratory.

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119 **Offshore experiments without mesocosms**

120 Studying impacts of anthropogenic stressors on marine animals in natural conditions
121 without the use of mesocosms is challenging and has only been performed on very few
122 factors such as sedimentation (e.g. Weber et al. 2006) and noise (Brandt et al. 2011, Vabø et
123 al. 2002). Studying impacts of anthropogenic stressors on animals in offshore experiments
124 allows investigations of wild free-ranging animals in their natural environment, including
125 regional environmental physicochemistry. The main disadvantages of offshore studies
126 include the inability to test stressors without permanently contaminating large areas (e.g.
127 when using chemicals) and difficulties in creating future stressor conditions (e.g. when
128 studying ocean acidification or warming) without the use of enclosures. If testing the focal
129 stressor is possible without using an enclosure, it is generally difficult to modify open-water
130 environments in a controlled manner or to control confounding variables during the study,

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3 131 such as nearby human activities or wave action. Continuous tracking of individuals across
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5 132 time can also be compromised due to the spatial area used by the animals of interest.
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7 133 Furthermore, during the stressor experience, free-ranging animals might leave the affected
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9 134 area as a result, eliminating the possibility to investigate long-term stressor exposure and
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11 135 potential habituation.
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17 137 **Novel approach: advantages and disadvantages of shipbuilding docks to study impacts of**
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19 138 **anthropogenic stressors**

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21 139 A setup that combines the advantages of the controlled environment of a laboratory with an
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23 140 ecologically-relevant large-scale marine arena would be ideal for studying impacts of
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25 141 anthropogenic stressors on marine organisms. In freshwater environments, whole lakes
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27 142 have been used to study impacts of anthropogenic stressors, including eutrophication
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29 143 (Schindler et al. 2008), pharmaceuticals (Kidd et al. 2014) and anthropogenic noise
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31 144 (Jacobsen et al. 2014). Lake experiments have led to substantial advances since they allow
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33 145 for: i) isolation of the stressor of interest and quantification of its impact on wild
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35 146 populations, ii) assessment of ecological risks at the population level, and iii) validation of
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37 147 responses of organisms observed in laboratory experiments with those seen in the field.
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39 148 Moreover, whole lake studies enable the study of entire ecosystems and allow
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41 149 characterization of, for example, natural behavior, pollutant levels, abundance and
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43 150 preferred distributions of organisms prior to and following introduction of the stressor.
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49 151 Whole environment approaches are generally not feasible in the marine
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51 152 environment. Flooded shipbuilding docks therefore provide a useful potential addition to
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53 153 existing methods. Shipbuilding docks are found around the world, with more than 410
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55 154 marine shipbuilding docks (>100 m length) currently in operation (Barnes et al. 2006), and
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3 155 many more that are fully functional but not in use. Although docks cannot fully replicate the
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5 156 marine environment, they offer many of the same advantages as whole-lake manipulations.
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7 157 For example, because of their considerable size, shipbuilding docks enable testing of how
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9 158 anthropogenic stressors impact on free-moving animals exhibiting natural behavior, and
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11 159 allow the use of large numbers of animals from a range of interacting species, thus creating
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13 160 experimental ecosystems. Docks can thus generate data on impacts of animal groups under
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15 161 semi-natural conditions, including complex group interactions and interspecific interactions.
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17 162 Docks also enable assessment of how individual impacts may scale up to community-level
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19 163 effects. On a practical level, docks allow complete drainage which greatly facilitates
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21 164 placement and retrieval of equipment (e.g. to position equipment that measures the
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23 165 stressor of interest), ensure good water quality, and enable recapture of all study animals.
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25 166 The main disadvantages of shipbuilding docks compared with offshore setups are the lack of
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27 167 natural environmental variation such as tides, and the absence of a typical coastal benthic
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37 170 [Box 1; suitability of using a shipbuilding dock to study biological impacts of noise](#)
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39 171 In our case study, we trialed the use of a flooded shipbuilding dock to study impacts of
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41 172 anthropogenic underwater noise on several marine fish species. Underwater noise is an
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43 173 internationally recognized pollutant (e.g. included in the US National Environmental Policy
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45 174 Act) and has been shown to impact a number of fish species (Slabbekoorn Hans et al. 2010).
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47 175 For example, noise exposure in fishes has been shown to increase stress, affect behavior,
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49 176 cause injury and mortality (Bruintjes and Radford 2013, Halvorsen et al. 2012, Hawkins et al.
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51 177 2014, Simpson et al. 2016, Vabø et al. 2002). Pile driving is commonly used during
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53 178 construction of offshore wind turbines and oil platforms, and generally creates loud and
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3 179 impulsive noise (>200 dB re 1 μ Pa at source, 1000s of strikes per day). We piloted the use of
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5 180 a former shipbuilding dock to investigate impacts of pile driving on fish behavior. Our
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7 181 particular interest focused on whether pile driving would result in avoidance behavior,
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9 182 changes in swimming speeds, and altered within-species interactions. These measures are
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11 183 particularly difficult to study in many experimental setups, due to the limited possibility to
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13 184 consider natural behavior, the lack of space to test multiple large fishes, the lack of space
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15 185 for an industrial-sized noise source, and general noise-propagation difficulties. As a
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17 186 consequence, there is a general lack of studies that investigate the impact of noise in large
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19 187 water bodies on wild marine animals using realistic anthropogenic noise sources.
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24 188 The dock used here is located at the Offshore Renewable Energy Catapult centre in
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26 189 Blyth, UK and provides a fully marine experimental arena. Draining and refilling of the dock
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28 190 can be performed within one day (>5 million liters). Dimensions of the dock are 93 x 18 m,
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30 191 with an average water depth of 3 m (range 2.5–3.5 m). The dock incorporated a simulated
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32 192 seabed consisting of sand and small stones from local North Sea sediment (3.5 m deep). At
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34 193 the seaward end of the dock, a section was retained at full depth without sediment (water
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36 194 depth 6.5 m) to facilitate draining. The simulated seabed was held in place by a concrete
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38 195 wall (Fig. 1) and a large net was placed inside the dock (mesh size: 15 mm) to retain study
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40 196 animals within the main simulated seabed section (resulting arena size 81 x 18 x 3 m).
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45 197 The noise, simulated impulsive impact piling, was generated using a hydraulic post-
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47 198 driver (Wrag Penna200, hammer weight 200 kg) mounted behind a tractor (Fig. 2). A steel
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49 199 pipe (7.5 m length, 16.5 cm diameter, 0.65 cm thickness), with a steel plate (151 x 164 x 1.4
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51 200 cm) welded 50 cm from the base to ensure a stable pile position during the piling trials, was
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53 201 used as an experimental pile. During trials, the post-driver hammer operated at 10 strikes
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per minute for a 2-h hour period twice a day for 2.5 successive days (animals were given two days to acclimatize to the dock prior to the noise trials). Sound pressure and particle velocity recordings of the pile-driving operation were made at 10 m intervals at two depths to allow for detailed characterization of the acoustic conditions with and without piling (for details of equipment see Simpson et al. 2016).

Movement patterns of wild-caught Atlantic cod (*Gadus morhua*), black seabream (*Spondyliosoma cantharus*) and plaice (*Pleuronectes platessa*) were assessed during pile driving using small positioning tags (16 x 4 mm; HTI, Seattle, USA) that were programmed to report individual positions every 2.5 s. The results demonstrate increased distance from the noise source during pile-driving exposure, suggesting avoidance, and increased swimming speed, suggesting increased stress levels, for cod and black seabream compared to ambient control conditions (see Fig. 3 for an example track of a cod before and during piling). Nearest-neighbor distance of cod and seabream was not impacted by pile-driving exposure. Plaice, which lack a swim bladder and thus cannot detect acoustic pressure, did not change movement or interactions during pile-driving exposure. (NB: Full details will be presented in Bruintjes *et al.* in prep.)

Using a flooded shipbuilding dock approach made it possible to investigate the impact of a real noise source (pile driving) on commercially important marine fishes. The sound gradient created by the pile driver made it possible to investigate potential avoidance responses of the study animals during exposure, a response that is impossible to study in the laboratory. The results indicate that pile-driving noise results in avoidance behavior by both cod and black seabream, without influencing nearest-neighbor distances.

225 Moving forward

226 Here, we present a novel and alternative way to upscale substantially controlled laboratory
227 experiments aimed at studying impacts of anthropogenic stressors on marine animals,
228 without the need to conduct experiments offshore, which might not be possible at all for
229 some stressors.

230 Our case study described in Box 1 – using a flooded shipbuilding dock to study
231 impacts of a global anthropogenic stressor (noise) on several marine fish species –
232 demonstrates that shipbuilding docks can be used successfully to study multiple wild-caught
233 animals simultaneously and to obtain information concerning: (1) individual and group
234 responses, (2) intraspecific interactions, (3) stressor avoidance, and (4) interspecific
235 differences in reaction to exposure of the same stressor. The dock results are not easily
236 obtained in small setups due to the typical challenge of keeping groups of fishes in small
237 spaces without impairing natural behavior, and the difficulty of creating a realistic stressor
238 gradient. Moreover, such results are challenging to obtain offshore using mesocosms due to
239 complicated logistics and lack of controlled experimental conditions, while studying impacts
240 of anthropogenic stressors on free-ranging animals offshore without the use of mesocosms
241 causes difficulties in tracking animals individually and thus obtaining information on
242 individual and group interactions.

243 We suggest that not only noise, but also impacts of other important anthropogenic
244 stressors could be tested using a dock setup, including stressors that cannot be easily
245 investigated offshore. For example, ocean warming could be studied through the use of
246 (multiple) heating devices; such studies could create a temperature-gradient that could give
247 essential information concerning preferred temperatures during various life stages, as well

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3 248 as impacts on free-moving animals during or following warming (Table 2). Additional
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5 249 anthropogenic stressors that could be studied using a dock approach, including unique
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7 250 opportunities and suggestions on how to perform the specific manipulations and
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9 251 corresponding challenges, are listed in Table 2.
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13 252 Moving forward, dock setups could help answer questions concerning the impacts of
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15 253 anthropogenic stressors at a community level through creating and studying mini-
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17 254 ecosystems inside the dock. Dock setups allow studying simultaneous large-scale and long-
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19 255 term exposure of stressors and their impact on free-moving marine animals, providing
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21 256 invaluable data concerning stressor impacts at environmentally relevant exposure levels and
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23 257 predicted future stressor levels. Such results provide essential parameters for predictive
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25 258 models on population, community and ecosystem level impacts. Statistical and mechanistic
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27 259 models have been successfully developed to predict biological responses to a range of
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29 260 environmental stressors, for example, species distributional changes due to ocean warming
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31 261 (Cheung et al. 2009) and acidification (Le Quesne and Pinnegar 2012), but these models are
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33 262 typically limited by a lack of accurate or realistic data for the species or stressor of interest.
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35 263 Ultimately, predictive models could help to assess current and future ecological risks and,
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37 264 although this might be complicated, help to facilitate appropriate management and develop
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39 265 suitable mitigation strategies.
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49 267 Conclusion

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51 268 Flooded shipbuilding docks could be a useful addition to the existing repertoire of methods
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53 269 used to study impacts of stressors on marine animals, especially since the approach allows
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55 270 investigations of anthropogenic stressors that are currently challenging to test in large-scale
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271 experiments. The dock approach overcomes many issues of laboratory-based and offshore
272 experiments and permits tight experimental control whilst allowing for control of many of
273 the confounding factors that operate in natural systems.

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369 Figure 1.

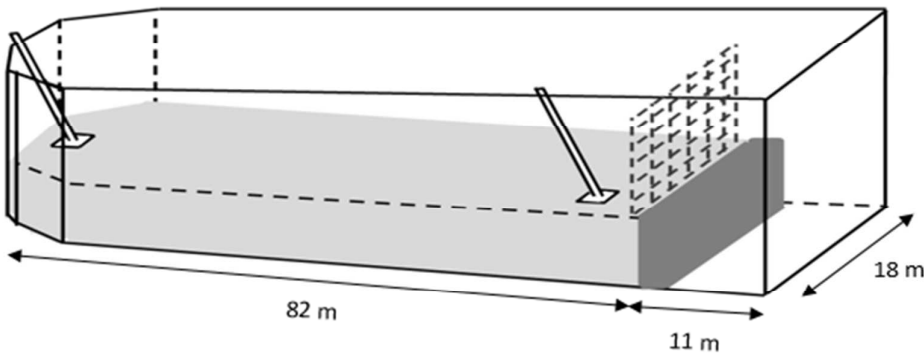


Figure 1: Schematic representation of the shipbuilding dock with the simulated seabed (light grey), the wall holding the seabed in place (dark grey), two piles, and the net keeping the animals in the simulated seabed section (the dashed parallelogram). The section lacking sediment at the right side was necessary for water drainage.

370

371 Figure 2.



372 Figure 2. The post-driver and pile set up.

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Figure 3.

a.

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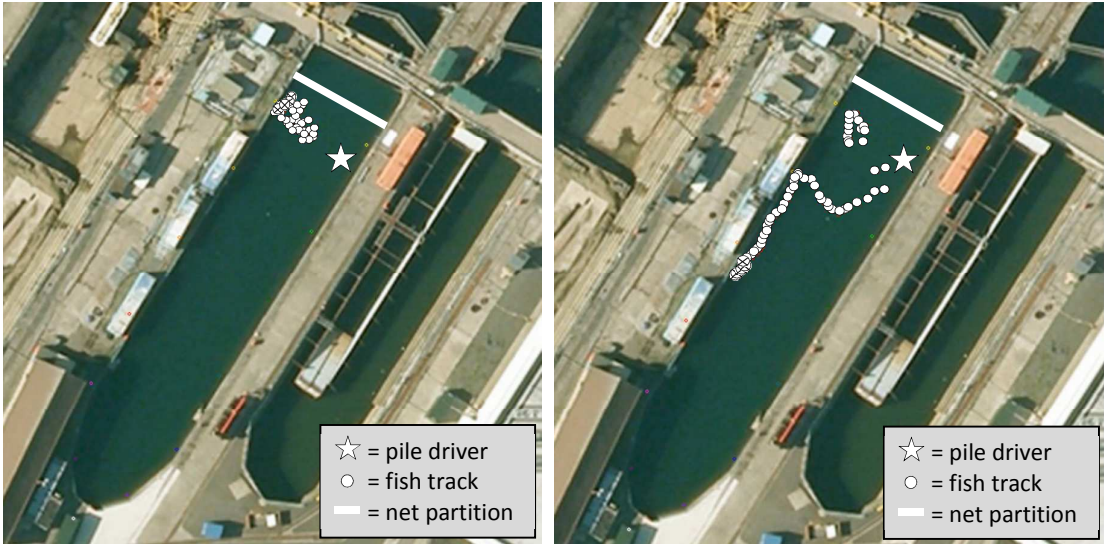


Figure 3. Aerial view of the dock with (a) the swim track (white dots) of a naïve cod during the five minutes prior to pile driving (ambient control), and (b) the swim track of the same fish during the first five minutes of pile driving. The white dots encircling an 'x' depict the last five time steps (base image from Google Maps).

381 **Table legends**

382

383 **Table 1.** Merits and limitations of studying impacts of anthropogenic stressors on marine
384 animals using aquaria in laboratory settings, shipbuilding docks and offshore setups (divided
385 into offshore using mesocosm [a structure that retains focal animals in one location] and
386 offshore using free ranging animals). ✓✓ = high agreement, ✓ = medium agreement, - = no
387 agreement nor disagreement, ✕ = medium disagreement, ✕✕ = high disagreement.

388

389 **Table 2.** Examples of anthropogenic stressors that could be studied using a shipbuilding
390 dock setup including their opportunities, how to perform the manipulations and challenges.
391 Stressors (in italic) are arranged in the following categories: global change, chemical,
392 ecological and multiple stressors (in bold).

Table 1.		Aquarium ¹	Ship-building dock	Offshore (meso-cosm) ²	Offshore (free ranging)
General experimental considerations	Explore potential future impact stressor	✓ ✓	✓ ✓	✓ ✓	✗ ✗
	Test stressor on a large scale	✗ ✗	✓	✓	✓ ✓
	Use free moving animals	-	✓	✓	✓ ✓
	Natural behaviour	-	✓	✓	✓ ✓
	Environmental complexity	✗ ✗	-	✓	✓ ✓
	Controlled experimental conditions	✓ ✓	✓	✗	✗ ✗
	Chronic exposure studies	✓ ✓	✓ ✓	✓ ✓	✗ ✗
	Ecosystem/community level experiments	✗	✓	✓	✓ ✓
	Long-term experiments	✓ ✓	✓	✓	✗ ✗
	Reality stressor experience	-	✓	✓ ✓	✓ ✓
	Stable stressor concentration/quantity (e.g. chemical compound, noise)	✓	✓	✗	✗ ✗
	Subjects only exposed to the stressor of interest	✓ ✓	✓ ✓	✗ ✗	✗ ✗
	Natural habitat (e.g. tides, food, water quality)	✗ ✗	-	✓	✓ ✓
	Ease of logistics (e.g. setup time, resource availability)	✓ ✓	-	✗	✗ ✗
	Test large organisms or those that need a large living space	✗ ✗	✓	✓	✓ ✓
	Ease of tracking individuals	✓ ✓	✓	✓	✗ ✗
Sound-specific experiments considerations	Straightforward real-time measuring	✓ ✓	✓	✗	✗ ✗
	Low cost	✓ ✓	✓	✗	✗ ✗
	Site security	✓ ✓	✓	✗ ✗	✗ ✗
	Use of real noise sources	✗	✓	✓	✓ ✓
	Sound propagation quality	✗ ✗	✓	✓ ✓	✓ ✓
	Natural sound pressure and particle motion levels	✗	✓	✓	✓

¹A 200 l aquarium example was used for comparison.

²A 20 x 20 x 2 m cage-like structure, i.e. allowing water flow but retaining focal animals, was used for comparison.

Table 2.**Opportunities****How to perform manipulation****Challenges**

Global Change stressors	<i>Ocean warming</i>	Gradient establishment (to study preferred temperatures)	Dock water temperature can be increased using heaters	Numerous heaters and high energy requirement
	<i>Acidification</i>	Gradient establishment	Dock water can be acidified using continuous CO ₂ injections	-Large CO ₂ quantities needed -CO ₂ exchange with atmosphere reduces levels
	<i>Salinity</i>	From fresh water to hypersaline	Modify dock water salinity using fresh water or salt	Establishing large quantities of water with certain salinity
	<i>Low dissolved oxygen levels (hypoxia)</i>	Gradient establishment	Low dissolved oxygen levels through continuous N ₂ /air mixture injections	Use of chemicals to drop oxygen levels
	<i>Sedimentation</i>	Gradient establishment	Sedimentation can be simulated through addition of e.g. fine sand.	Large sedimentation quantities needed; sedimentation addition issues
Chemical stressors	<i>Chemicals (including pharmaceuticals)</i>	-Gradient establishment -Possibility to study impact of water-soluble and non-soluble chemicals	Contaminate dock water and/or the dock sediment using (biodegradable) chemicals or pharmaceuticals	Non- and slow-biodegradable chemicals need filtering out following experiments, which might be difficult and costly
	<i>Eutrophication</i>	Establishment of a gradient	Dock water nutrient levels can be enriched using fertilizer or phosphates	Large quantities of fertilizer needed
Ecological stressors	<i>Light</i>	-Gradient establishment -Studies on e.g. avoidance and bio-rhythm impact -Use of submerged and non-submerged light	Dock water can be lit using aerial or submersible floodlights	Powerful lights required
	<i>Invasive species</i>	-Impacts of invasive species on local animals or communities -Identification of vulnerable life stages	Introduce invasive species	-Transport of invasive species to the site -removal of invasive species to avoid subsequent release
	<i>Noise</i>	Gradient establishment	Use a noise source inside the dock (e.g. a pile driver [Fig. 3] or airgun; see Box 1 for a noise experiment in a shipbuilding dock)	Construction of proper noise source
Multiple stressors	<i>Combination of stressors</i>	Collection of data on the impacts of multiple pollutants simultaneously	Use different combinations of anthropogenic stressors	Simultaneous exposure of the study objects to both stressors

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